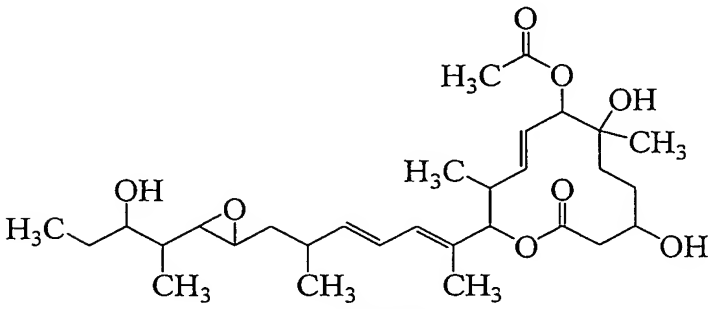
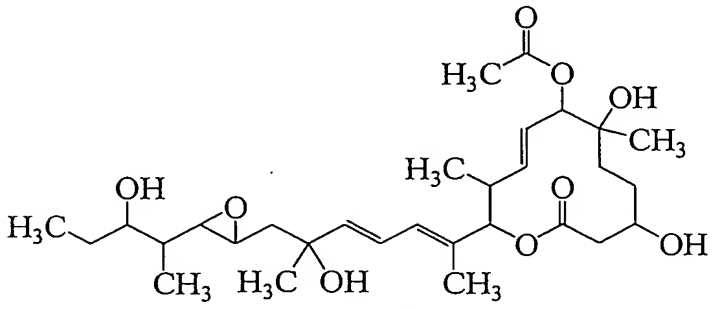
Claims

1. A DNA participating in biological transformation of a macrolide compound (hereinafter referred to as a macrolide compound 11107B) represented by the formula (I):



11107B (I)

into a 16-position hydroxy macrolide compound represented by the formula (II):



11107D (II),

the DNA being an isolated and pure DNA comprising a DNA encoding a protein having 16-position hydroxylating enzymatic activity or ferredoxin, partly or entirely or its variant.

2. The DNA according to Claim 1, which is characterized by the following (a), (b) or (c):

(a) a DNA encoding a protein having the enzymatic activity in hydroxylating the 16-position of the macrolide compound 11107B

and selected from the group consisting of a continuous nucleotide sequence from the base 1322 to base 2548 of the sequence No. 1; a continuous nucleotide sequence from the base 420 to base 1604 of the sequence No. 2; and a continuous nucleotide sequence from the base 172 to base 1383 of the sequence No. 3;

(b) a DNA which is a variant of the DNA described in the above (a);

(i) is hybridized with the DNA described in the above (a) under a stringent condition; and

(ii) encodes a protein having enzymatic activity in hydroxylating the 16-position of the macrolide compound 11107B; and

(c) a DNA encoding a protein having the same amino acid sequence as the protein encoded by the DNA described in the above (a) or (b) though it is not hybridized with the DNA described in the above (a) under a stringent condition because of the degeneracy of a gene codon.

3. A protein encoded by the DNA as claimed in Claim 2.

4. A self-replicative or integrating replicative recombinant plasmid carrying the DNA as claimed in Claim 2.

5. A transformant into which the recombinant plasmid as claimed in Claim 4 transforms.

6. A method of isolating a DNA encoding a protein having enzymatic activity in hydroxylating the 16-position of the macrolide compound 11107B, the method characterized by using the DNA as claimed in Claim 2 or a DNA constituted of a part of the DNA as a probe or a primer.

7. The DNA according to Claim 1, which is characterized by the following (d), (e) or (f):

(d) a DNA encoding ferredoxin and selected from the group consisting of a continuous nucleotide sequence from the base 2564 to base 2761 of the sequence No. 1, a continuous nucleotide sequence from the base 1643 to base 1834 of the sequence No. 2 and a continuous nucleotide sequence from the base 1399 to base 1593 of the sequence No. 3;

(e) a DNA which is a variant of the DNA represented by the above (d);

(i) is hybridized with the DNA described in the above (d) under a stringent condition; and

(ii) encodes a protein having a ferredoxin function; and

(f) a DNA encoding a protein having the same amino acid sequence as the protein encoded by the DNA represented by the above (d) or (e) though it is not hybridized with the DNA described in the above (d) under a stringent condition because of the degeneracy of a gene codon.

8. A protein encoded by the DNA as claimed in Calim 7.

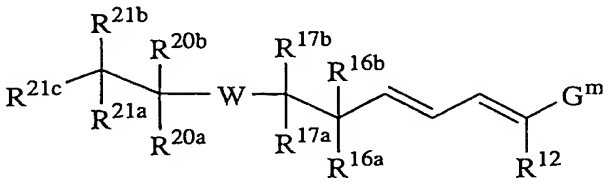
9. A self-replicative or integrating replicative recombinant plasmid carrying the DNA as claimed in Claim 7.

10. A transformant into which the recombinant plasmid as claimed in Claim 9 transforms.

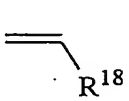
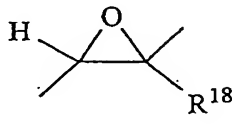
11. A method of isolating a DNA encoding a protein having a ferredoxin function, the method characterized by using the DNA as claimed in Claim 7 or a DNA constituted of a part of the

DNA as a probe or a primer.

12. A method of producing a 16-position hydroxy macrolide compound, the method comprises the steps of culturing the transformant as claimed in Claim 5 or 10 in a medium; bringing the proliferated transformant into contact with a macrolide compound represented by the formula (III):



(III)

(wherein W represents  .or.  ;

R^{12}, R^{16b}, R^{17a}, R^{17b}, R^{18}, R^{20a}, R^{20b}, R^{21a} and R^{21b}, which may be the same as or different from, respectively represent:

(1) hydrogen atom;

(2) a C_{1-22} alkyl group which may have a substituent;

(3) -OR (wherein R represents:

1) hydrogen atom; or

2) a C_{1-22} alkyl group;

3) a C_{7-22} aralkyl group;

4) a 5-membered to 14-membered heteroaryloxyalkyl group;

5) a C_{2-22} alkanoyl group;

6) a C_{7-15} aroyl group;

7) a C_{3-23} unsaturated alkanoyl group;

8) $-COR^{co}$ (wherein R^{co} represents:

8-1) a 5-membered to 14-membered heteroaryloxyaryl group;

8-2) a C_{1-22} alkoxy group;

8-3) an unsaturated C_{2-22} alkoxy group;

8-4) a C_{6-14} aryloxy group;

8-5) a 5-membered to 14-membered heteroaryloxy group; or

8-6) a 3-membered to 14-membered nitrogen-containing non-aromatic heterocyclic group, each of which may have a substituent);

9) a C_{1-22} alkylsulfonyl group;

10) a C_{6-14} arylsulfonyl group; or

11) $-SiR^{s1}R^{s2}R^{s3}$, (wherein R^{s1}, R^{s2} and R^{s3}, which may be the same as or different from, respectively represent a C_{1-6} alkyl group or a C_{6-14} aryl group), each of which may have a substituent);

(4) a halogen atom; or

(5) $-R^{M}-NR^{N1}R^{N2}$, {wherein R^{M} represents a single bond or -O-CO-; and R^{N1} and R^{N2}

1) may be the same as or different from, respectively represent:

1-1) hydrogen atom; or

1-2)

(i) a C_{1-22} alkyl group;

(ii) an unsaturated C_{2-22} alkyl group;

(iii) a C_{2-22} alkanoyl group;

(iv) a C_{7-15} aroyl group;

(v) an unsaturated C_{3-23} alkanoyl group;

(vi) a C_{6-14} aryl group;

(vii) a 5-membered to 14-membered heteroaryl group;

(viii) a C_{7-22} aralkyl group;

(ix) a C_{1-22} alkylsulfonyl group; or

(x) a C_{6-14} arylsulfonyl group, each of which may have a substituent, or

2) and R^{N1} and R^{N2} may be combined with the nitrogen atom to which they bound, to form a 3-membered to 14-membered nitrogen-containing non-aromatic heterocyclic group}, provided that

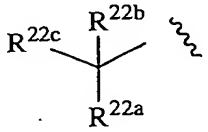
R^{21a} and R^{21b} may be combined with each other to form (i) a ketone structure (=O) or (ii) an oxime structure {=NOR^{ox} (wherein R^{ox} represents a C_{1-22} alkyl group, an unsaturated C_{2-22} alkyl group, a C_{6-14} aryl group, a 5-membered to 14-membered heteroaryl group or a C_{7-22} aralkyl group, each of which may have a substituent)};

R^{16a} represents hydrogen atom;

R^{21c} represents:

(1) hydrogen atom; or

(2)



(wherein R^{22a}, R^{22b} and R^{22c}, which may be the same as or different from, respectively represent:

1) hydrogen atom;

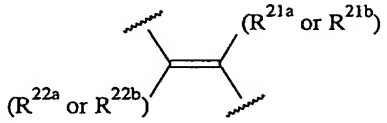
2) a C_{1-6} alkyl group;

3) -OR (wherein R has the same meaning as the above);

4) $-R^{M}-NR^{N1}R^{N2}$ (wherein R^{M}, R^{N1} and R^{N2} have the same meanings as the above); or

5) a halogen atom, or

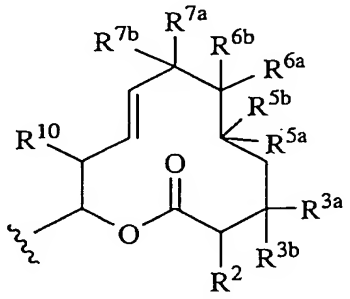
any one of R^{21a} and R^{21b} may be combined with any one of R^{22a} and R^{22b} to form the partial structure;



); and

G^{m} represents:

(1) a group represented by the formula (GM-I):



(GM-I)

{wherein

R^{2} and R^{10}, which may be the same as or different from , respectively represent hydrogen atom or a C_{1-22} alkyl group;

R^{3a}, R^{3b}, R^{5a}, R^{5b}, R^{6a} and R^{6b}, which may be the same as or different from, respectively represent:

1) hydrogen atom;

2) hydroxyl group;

3)

3-1) a C_{1-22} alkyl group;

3-2) a C_{1-22} alkoxy group;

3-3) a C_{6-14} aryloxy group;

3-4) a 5-membered to 14-membered heteroaryloxy group;

3-5) a C_{2-22} alkanoyloxy group;

3-6) a C_{7-15} aroyloxy group;

3-7) a C_{3-23} unsaturated alkanoyloxy group;

3-8) $-OCOR^{co}$ (wherein R^{co} has the same meaning as the above);

3-9) a C_{1-22} alkylsulfonyloxy group;

3-10) a C_{6-14} arylsulfonyloxy group; or

3-11) $-OSiR^{s1}R^{s2}R^{s3}$ (wherein R^{s1}, R^{s2} and R^{s3} have the same meanings as the above), each of which may have a substituent;

4) a halogen atom; or

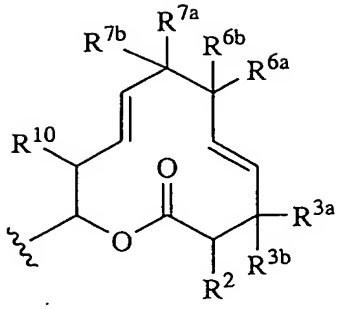
5) $-R^{M}-NR^{N1}R^{N2}$ (wherein R^{M}, R^{N1} and R^{N2} have the same meanings as the above); or

R^{5a} and R^{5b} may be combined with each other to form a ketone structure (=O); or

R^{6a} and R^{6b} may be combined with each other to form a spirooxysilanyl group or an exomethylene group; or

R^{7a} and R^{7b}, which may be the same as or different from, respectively represent hydrogen atom or $-OR^{H}$ (wherein R^{H} represents hydrogen atom, a C_{1-22} alkyl group or a C_{2-22} alkanoyl group)};

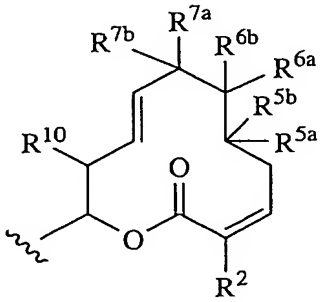
(2) a group represented by the formula (GM-II):



(GM-II)

(wherein R^2, R^{3a}, R^{3b}, R^{6a}, R^{6b}, R^{7a}, R^{7b} and R^{10} have the same meanings as those in the formula (GM-I));

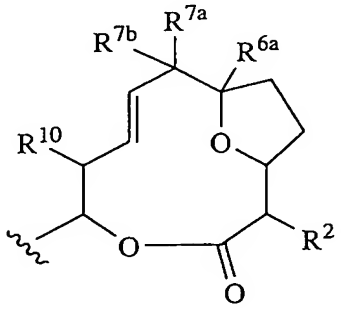
(3) a group represented by the formula (GM-III):



(GM-III)

(wherein R^2, R^{5a}, R^{5b}, R^{6a}, R^{6b}, R^{7a}, R^{7b} and R^{10} have the same meanings as those in the formula (GM-I));

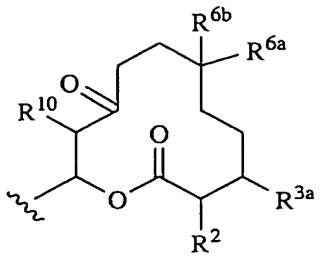
(4) a group represented by the formula (GM-IV):



(GM-IV)

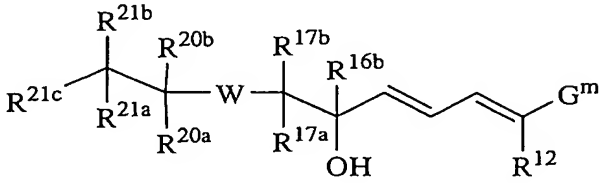
(wherein R^2, R^{6a}, R^{7a}, R^{7b} and R^{10} have the same meanings as those in the formula (GM-I)); or

(5) a group represented by the formula (GM-V):



(GM-V)

(wherein R^2, R^{3a}, R^{6a}, R^{6b} and R^{10} have the same meanings as those in the formula (GM-I))) during or after culturing, to convert it into a 16-position hydroxy macrolide compound represented by the formula (IV):

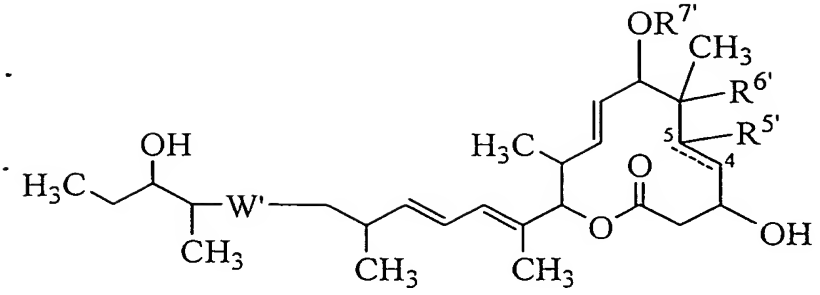


(IV)

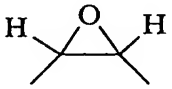
(wherein W, R^{12}, R^{16b}, R^{17a}, R^{17b}, R^{20a}, R^{20b}, R^{21a}, R^{21b}, R^{21c} and G^m have the same meanings as those in the formula (III)); and then collecting the 16-position hydroxy macrolide compound thus converted.

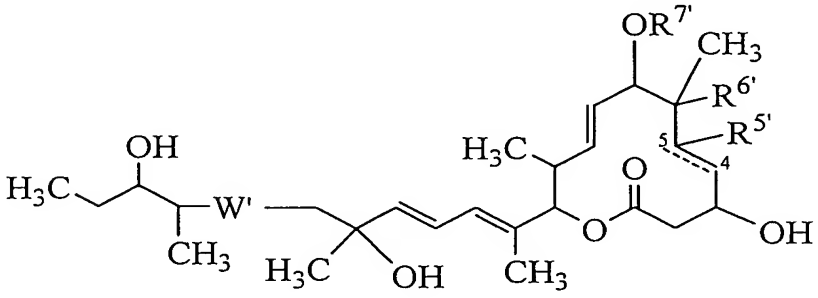
13. A production method according to Claim 12, wherein the transformant is the transformant as claimed in Claim 5 and has a DNA encoding ferredoxin.

14. The production method according to Claim 12, the method comprises the step of converting a compound represented by the formula (III-a):



(III-a)

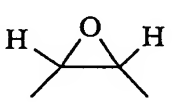
(wherein $^{5}\!=\!\!=\!\!=\!^{4}$ represents a double bond or a single bond; W' represents a double bond or  ; $R^{5'}$ represents hydrogen atom or an acetoxy group; $R^{6'}$ represents hydrogen atom or hydroxyl group; and $R^{7'}$ represents hydrogen atom or acetyl group) into a compound represented by the formula (IV-a):



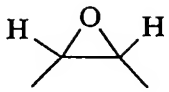
(IV-a)

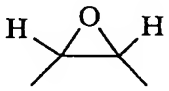
(wherein $^{5}\!=\!\!=\!\!=\!^{4}$, W', $R^{5'}$, $R^{6'}$ and $R^{7'}$ have the same meanings as those in the formula (III-a)).

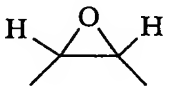
15. The production method according to Claim 14, wherein, in the conversion of the compound of the formula (III-a) into the compound of the formula (IV-a), the compound to be subjected is a compound selected from the group consisting of:

(1) a compound in which $^{5}\!=\!\!=\!\!=\!^{4}$ is a single bond; W' is  ;

and $R^{5'}$, $R^{6'}$ and $R^{7'}$ are respectively hydrogen atom;

(2) a compound in which $5^{---}4$ is a single bond, W' is  ; $R^{5'}$ and $R^{6'}$ are respectively hydrogen atom; and $R^{7'}$ is acetyl group;

(3) a compound in which $5^{---}4$ is a single bond, W' is  ; $R^{5'}$ and $R^{7'}$ are respectively hydrogen atom; and $R^{6'}$ is hydroxyl group;

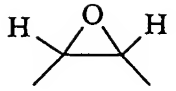
(4) a compound in which $5^{---}4$ is a single bond, W' is  ; $R^{5'}$ is hydrogen atom, $R^{6'}$ is hydroxy group; and $R^{7'}$ is acetyl group;

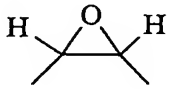
(5) a compound in which $5^{---}4$ is a single bond; W' is a double bond; and $R^{5'}$, $R^{6'}$ and $R^{7'}$ are respectively hydrogen atom;

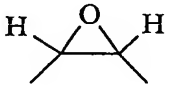
(6) a compound in which $5^{---}4$ is a single bond; W' is a double bond; $R^{5'}$ and $R^{6'}$ are respectively hydrogen atom; and $R^{7'}$ is acetyl group;

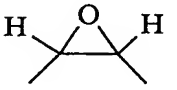
(7) a compound in which $5^{---}4$ is a single bond; W' is a double bond; $R^{5'}$ and $R^{7'}$ are respectively hydrogen atom; and $R^{6'}$ is hydroxyl group;

(8) a compound in which $5^{---}4$ is a single bond; W' is a double bond; $R^{5'}$ is hydrogen atom; $R^{6'}$ is hydroxy group; and $R^{7'}$ is acetyl group;

(9) a compound in which ^{5}=^{4} is a double bond; W' is  ;
$R^{5'}$ and $R^{7'}$ are respectively hydrogen atom; and $R^{6'}$ is hydroxyl group;

(10) a compound in which ^{5}=^{4} is a double bond; W' is  ;
$R^{5'}$ is hydrogen atom; $R^{6'}$ is hydroxy group; and $R^{7'}$ is acetyl group;

(11) a compound in which ^{5}—^{4} is a single bond; W' is  ;
$R^{5'}$ is acetoxy group; $R^{6'}$ is hydroxyl group; and $R^{7'}$ is hydrogen atom; and

(12) a compound in which ^{5}—^{4} is a single bond; W' is  ;
$R^{5'}$ is an acetoxy group; $R^{6'}$ is hydroxyl group; and $R^{7'}$ is acetyl group.

16. Use of the transformant as claimed in Claim 5 or 10 for producing a 16-position hydroxy macrolide compound.